

**Evaluation of Liver Biomarkers among Type Two Diabetic Patients with  
and without Chronic Kidney Disease Attending Jimma Medical Center,  
Jimma southwest, Ethiopia, 2023**



Institute of Health

Faculty of Medical Sciences

Department of Biomedical Sciences

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in Partial Fulfillment of the Requirements for the Degree of Masters of Science  
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Jimma, Ethiopia

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## Abstract

**Background:** - Diabetes mellitus is an abnormal metabolic condition manifested by high blood sugar levels resulting from defective insulin action or, secretion, or both. Liver disorder is known to be one of the risk factors for diabetes and diabetics are known to be prone to both liver and kidney disorders. But, the association between these diseases is not well studied.

**Objective:** The study was aimed to evaluate liver biomarkers and their associated factors among T2D patients with CKD and T2D without CKD attending JMC from November 01, 2022 to January 20, 2023.

**Materials and Methods:** - An institutional -based comparative cross-sectional study design was implemented at Jimma Medical Center from November 01, 2022 to January 20, 2023. Consecutive sampling technique was implemented to gather the data. The data were entered into EpiData version 4.6.0.6, exported to SPSS version 26 for analysis, and statistically tested at a 95% Confidence level. The mean difference was tested using an independent t-test and the relationship predictors and dependant variables were tested by using logistic regression.

**Results:** A Total of 142 of type 2 diabetes subjects were included in the study. Mean value of alkaline phosphatase was higher in type2 with chronic kidney disease ( $115.27 \pm 22.41$  vs.  $97.42 \pm 29.57$   $P < 0.001$ ) but that of total bilirubin ( $0.86 \pm 0.47$  vs.  $1.03 \pm 0.48$ ,  $p = 0.028$ ,) and direct bilirubin ( $0.16 \pm 0.083$  vs.  $0.21 \pm 0.09$   $p = 0.003$ ), were lower among T2D patients with CKD. T2D patients with chronic kidney disease were 2.2 times likelihood to have elevated alkaline phosphatase levels than diabetics without chronic kidney disease (AOR =2.2 (1.09, 4.46)  $p = 0.028$ ). T2D patients without chronic kidney disease were 2.8 and 5.1 times likelihood to have elevated direct and total bilirubin than T2D with chronic kidney disease (AOR = 2.8 (1.29, 6.06)  $P = 0.009$  and AOR = 5.14(2.24, 11.56)  $P < 0.001$ ) respectively.

**Conclusion:** The mean of alkaline phosphatase was significantly higher in type 2 diabetes with chronic kidney disease than without chronic kidney disease. Whereas the mean of direct and total bilirubin was lower in type 2 patients with chronic kidney disease.

**Recommendation:** stakeholders are recommended to consider liver biomarkers of type 2 patients with chronic kidney disease for early diagnosis, and prevention of liver disease.

**Key words:** Chronic kidney disease; Diabetes; liver Biomarkers

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## Abbreviations and Acronyms

ACR: Albumin Creatinine Ratio

ALP: Alkaline phosphatase

ALT: Alanine Aminotransferase

AST: Aspartate Aminotransferase

BMI: Body Mass Index

CKD: Chronic Kidney Disorder

CVD: Cardiovascular Disease

DBIL: Direct Bilirubin

DKD: Diabetic Kidney Disease

DM: Diabetes Mellitus

DM-CKD: Diabetes mellitus patients with Chronic Kidney Disorder

EGFR: Estimated Glomerular Filtration Rate

ESRD: End-Stage Renal Disease

JMC: Jimma Medical Center

LDH: Lactate Dehydrogenase

LFT: Liver Function Test

MAFLD: Metabolic Associated Fatty Liver Disease

MetS: Metabolic Syndrome

NAFLD: Non-Alcoholic Fatty Liver Disease

NASH: Non-Alcoholic Steatosis Hepatitis

T2D: Type 2 Diabetes

TBIL: Total Bilirubin

## Introduction

### 1.1. Background

Diabetes mellitus (DM) is a chronic disorder of metabolic characterize by long-lasting hyperglycemia, which comes from abnormal insulin action, secretion, or both (1). Type2 diabetes mellitus is known to be the main etiology of end-stage kidney disease (ESRD), non-traumatic lower-extremity impairment , and adulthood visual disability (2). Chronic kidney diseases (CKD) is defined as a gradual loss of renal function taking place over many months to years (3). This could be defined by abnormalities of renal structure and its performance for more than 3 months with an estimated glomerular filtration rate (eGFR) fewer than 60mL/min/1.73 m<sup>2</sup> and/ or others evidence of renal injury, like persistent albuminuria, defined as more than 30 mg of albumin in the urine per gram of urine creatinine (4).

Chronic kidney disease is the major complication of Type 2 diabetes mellitus (T2D) with 25–40% of T2D had renal complications (5). Chronic kidney disorder creates significant health problems in society as it causes increased mortality, morbidity, as well as economic burden. Several studies have been implement to identify risk factors which can predict real disease development in DM patients (6). Even though long DM duration of follow up, high blood pressure , dyslipidemia, and uncontrolled glycemic level have been detected as well-known risk factors for CKD in diabetic kidney disease, may researches are needed to be done to clarify pathophysiological mechanisms resulting in to CKD of T2D patients beyond the cultural risk factors (7).

The term ‘liver function tests’ frequently utilized as the commonly performed tests but, none of these tests are truly evaluate all functions of the liver. They are really biochemical indicators of hepatobiliary damage like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBIL) (8). Serum ALT is identified to reveal association with fat liver (9). The larger the serum ALT, the higher the likelihood of liver disease (10). Confirmed liver disease is found to be aggravated diabetic kidney disease (DKD) (11). This relation could be associated with a large prevalence of both disorder or an independent presence (10). However, liver disease and CKD have common risk factors, like central obesity, insulin resistance, atherogenic dyslipidemia, and high blood pressure (12). Moreover, researches have consistently approved that liver disease

is directly related with a larger prevalence of DKD while T2D is an important risk factor for both liver disease and CKD (13). However, prevalence of CKD is larger in liver disease patients, without DM status, when compared to patients without liver disease (50% vs. 5–30%) (14). Even early renal disorder was reported to be associated with more severe liver histology among biopsy-confirmed liver disease patients, describing the significance of early identifying for timely control of renal disease among T2D (15). In 2019, the American Diabetes Association recommended that patients with T2D with elevated plasma ALT stages or steatosis on liver ultrasound should be evaluated for Non-Alcoholic Steatosis Hepatitis (NASH) and liver fibrosis (16). Study examined that, the association among liver enzymes in the serum and progression of diabetes among whole Japanese population; in both sexes, the age-adjusted incidence of DM raised significantly with increasing quartiles of ALT in serum (17).

Type2 DM Patients who are at ESRD frequently have disorder of enzymes in serum due to two important reasons: (a) impaired removal and (b) existence of comorbidities. Among such enzymes, those liver enzymes own a significance role in that respect (18). Increased ALT in the serum levels were associated to larger remission/regression likelihood of DKD through preserved eGFR. In this relationship, ALT in the serum was rarely taken into accounts as a marker which indicates kidney function (19). Alternatively, ALT in the serum is understood to reduce with the increased severity of CKD (20). Liver disease was related to an elevated prevalence of CKD among individuals with T2D (21).

## 1.2. Statement of the Problem

Diabetes is an important cause of disability and death around the world and is a major risk factor for other diseases (22). Chronic kidney disease is a complex, growing chronic disease which is defined by either disorder of renal structure or function for > 3 months of severe indication for health, with markers of kidney injury or lowered glomerular filtration rate could be present (10). There is an initial phase of diabetic kidney condition called incipient diabetic nephropathy manifested by elevated albumin excretion within a range of 30-300mg/day. This level of disease can progress to overt nephropathy unless it treatment early, and eventually such patients develop ESRD (23).

Liver disease and chronic renal disease were related with poor prognosis and expensive in terms of costs; they have been main health problems in the societies owing to their elevating prevalence as well as incidence (8). In generally, liver disease have impact up to 25–30% (24) and CKD attack around 10–15% of whole population in adulthood age worldwide (24). Greater than one-third of people with liver disease have abnormal kidney function and impaired kidney function in patients with liver disorder is also related with the severity of liver impairment and the existence of T2D (25). Diabetes mellitus patients with liver disorder had higher aminotransferases levels and significant. However, ALP levels indicate no significant difference (26).

In 2019, DM and CKD had been the seventh highest non-communicable disorder, the 4<sup>th</sup> major cause of the death, and the 6<sup>th</sup> leading causative agent of disability globally (27). In the similar year, incidence cases, were 2.62 million, 134.58 million prevalence, and 405.99 thousand deaths due to DM- CKD comorbidities globally (28). Further studies reveal that T2D-CKD holds about 95.32, 96.27, and 83.20% of a total DM-CKD incident cases, disease and deaths respectively, indicating the crucial role of T2D in CKD progression (27). Burden of DM-CKD different among varied regions and countries (27). The magnitudes of DM-CKD in Africa ranges from 11% in Tunisia and 34.7% in Morocco (28). In Ethiopia, the prevalence of CKD in T2D is estimated to be 17.3% (2,29,30). However the prevalence of CKD in Jimma is 26% (3).

A limited number of researches have investigated the association between LFT and CKD as well as DKD patients in the world (20). To the best of our knowledge, the relationship between liver and renal diseases in patients with DM is not well-studied in the Ethiopian

population. Such information on the relationships between these chronic diseases and the possible risk factors could improve patient care practices, such as delaying the onset or progression of liver disorders and/or renal diseases in patients with DM. Therefore, this study was conducted to assess liver biomarkers including ALT, AST, ALP, DBIL, and TBIL among DM-CKD patients attending Jimma Medical Center (JMC).

### 1.3. Significance of Study

Evaluation of liver biomarkers among DM patients with and without CKD in Ethiopia is important for several reasons. These may include: increasing burden of the disease across the world as well as in the local population of Ethiopia, largely unknown state of T2D with CKD comorbidity care in the country and most patients with liver disease have no any form of symptom of the liver disorder and frequently are diagnosed when abnormal liver studies are stated on routine laboratory evaluation. Studies have been done on liver biomarkers among DM and CKD patients separately but there is little information on T2D with CKD comorbidity. However, there has been limited information on the association between liver biomarkers and T2D-CKD comorbidity patients in Ethiopia. Therefore, this study firstly, might improve patient care practices and early prevention, such as may help by delaying the onset or progression of liver disorders and/or renal diseases in patients with T2D. It could also serve as a baseline study for more interventional and/or advanced, multi-center studies.

## 2. Literature Review

### 2.1. Overview of Diabetes Mellitus and Chronic Kidney Disease

Diabetes mellitus is a complex disorder of abnormality in carbohydrates, lipids, and proteins metabolism defined by long lasting hyperglycemia (31). In 2021, there is a 463 million (9.3%) prevalence of DM and it is expected to rise to 700.2 million by 2045 worldwide (32). An epidemic progression of DM has taken place in developing countries of which 75% of Patients with DM live in low to middle-income countries where DM impact the productive age in countries (31).

Chronic kidney disease in diabetic patients is the most common diabetic complication worldwide, occurs in about 40 % of patients with diabetes, and become the most common cause of end-stage renal disease (ESRD) and renal replacement treatment due to the elevated prevalence of diabetes mellitus (29). Where existence of urinary Albumin-to-Creatinine Ratio (ACR) is normal or very mildly increased and eGFR above 60 ml/min/1.73 m<sup>2</sup>, the risk of progression to ESRD is very small and such individual frequently don't follow regular surveillance (33). Abnormal albuminuria is strongly related to microvascular injury in kidney glomeruli of DM and is a major microvascular complication in DM, decreased eGFR values are strongly associated to macrovascular (34).

The large vascular disorder is strongly related with cardiovascular risk factors which coexist with metabolic syndrome (MetS), including high blood pressure, atherogenic dyslipidemia, elevated low-density lipoprotein, cholesterol ingredients, leads to CKD (10). In 2015, greater than one of 10 adults were suffering from CKD and more than 600 million CKD worldwide (35) hypertension (61.25%) and diabetes (35.93%) were the two predominant risk factors related to CKD in our series (35).

Diabetic kidney disease develops in 40% of patients with T2D (36). A study indicates that the Prevalence of CKD in DM patients was 39.6% (37). Other Study found that approximately most of the patients with T2D, visiting the diabetic centers had diabetic nephropathy, in line with the distribution of urea, creatinine, and glomerular filtration rate (GFR) for group T2D was considerably different (38). Diabetes mellitus is the main cause of CKD and ESRD, particularly within developed countries and few developing nations as well, 20-40% of DM patients are complicated with CKD, OF which consists of 20-30% of all CKD patients (39).

Diabetic kidney disease is related to insulin resistance and alteration of liver biomarkers in addition to disorders in the red blood cell count and red blood cell shape that warrant usual monitoring amongst patients with diabetic kidney disorder (40). Greater than one-third of the patients with liver disease have affected kidney function and its prevalence in the patients with liver disease is based on the severity of the liver disease and the existence of DM (41). In diabetic patients with liver disease, ALP is a liver disease related marker while elevated, and was also significantly related to impaired renal function (42). Surprisingly, ALP is associated to the release of proinflammatory cytokines from the liver that are known to disrupt the glomerular endothelial glycocalyx, main to albuminuria, which may explain why ALP is a capacity surveillance marker in patients with NAFLD who are at chance for developing CKD (43). Moreover, elevated serum enzymes are related to an elevated risk of CKD (44).

Numerous studies have concluded that there is a lower in level of serum aminotransferases in patients with CKD compared to the normal population(41). Study reports that ALT and AST levels in serum tend to remain smaller in ESRD patients when compared to a normal people, and levels similarly reduce with the worsening (40). But, epidemiological proof suggests that liver disease is an independent risk for albuminuria, and the severity of steatosis is related with prevalence of albuminuria (45). Numerous cross-sectional researches investigated that the prevalence of CKD was much greater in patients with biopsy-confirmed NAFLD/NASH than in those without it (46). The pathophysiological mechanism for the reduction the serum aminotransferase levels in patients with CKD stays debatable. Elevated liver enzymes are indicative of NAFLD, which is characterized by fat accumulation in the liver (47). The possible mechanisms consist of a decreased in pyridoxal-5-phosphate that is a coenzyme of both ALT and AST, the existence of UV absorbing compound, and large levels of uremic chemical toxins and are because of water retention and hem dilution in patients of CKD. A study showed that elevated serum concentrations of ALT, AST, and ALP were positively related to an elevated risk of MetS and DM as well (48). Moreover, there was a positive association between elevated levels of LDH and CKD (49).

## 2.2 Factors Associated with Liver Biomarkers Disorder

### 2.2.1 Socio-Demographic Characteristics

Aging is a well-known risk factor involving several system and organ of the body which include kidney and liver, which has become health problem in public, specifically in developed countries. Even though liver disease was not independently associated to a research conducted by NEHASE indicated that age, high blood pressure, DM, high uric acid levels, and ALP were independent risk factors to CKD (50). Others Study show that diabetics with CKD were older with longer diabetes duration of follow-up (30,40,51). Additionally, another study declared that older age was known to become the independent factor of reduced estimated GFR and has been statically related with elevated incidence of CKD in diabetic patients (4). Study reported that Male sex was associated with the development of microalbuminuria (52).

### 2.2.2 Medical and Behavioural Factors

High blood pressure is another public health problem that strongly related predictor for CKD in all detected subjects (53). High blood pressure is a commonly affect nearly whole human systems including kidney which cause CKD in diabetic patients (54). Findings in research provided further evidence suggesting that elevated levels of liver enzymes (ALT, AST, and ALP) can be related to the risk of non-hepatic diseases, including, Hypertension (HTN) (48). Studies strongly suggest that hypertension is a well-established risk predictor in diabetic nephropathy (40). Systolic and diastolic blood pressure, were significantly highest among the overt nephropathy group, followed by microalbuminuria and normoalbuminuric subjects (51). Another study found that alcohol consuming, and smoking cigarette can further elevate the odds in population with elevated ALT levels (55). Additionally, AST and ALT levels were significantly affected by smoking (56).

### 2.2.3 Physical and Anthropometric Measurement

A Study reveals that the prevalence of elevated liver enzymes was significantly higher in the patients with higher BMI. Aminotransferase, particularly ALT has been proven to be related to general obesity (57). A significant correlation has been reported between increased ALT and WC in Korean adults (57). Among Australian adults, BMI had been located to be strongly associated with ALT (58). The mean value of measured systolic and diastolic blood pressure was higher among subjects with microalbuminuria and overt nephropathy compared with the normoalbuminuric group (51). On the other hand, both the lower and upper of BMI of  $\leq 25$  and  $\geq 30$  kg/m<sup>2</sup> have significant variation among total diabetic nephropathic patients than

those without nephropathic patients (59). Study found that overweight or central obesity elevate the odds in the people with elevated ALT levels (55).

#### 2.2.4 Biochemical Measurement

Routine LFTs generally consist of serum concentrations of ALT, AST, ALP, TBIL, and DBIL) (60). ALT is localized within the hepatic cell cytosol, while AST is found largely within the mitochondria (53). Biomarkers of liver fibrosis correlated well with the prevalence of DM and CKD, specifically AST and ALT, confirming similar findings reported within the general NAFLD population (60). Specifically, liver enzymes, such as ALT and AST levels are increased. But, the enzymes may not be increased in whole cases of liver disease and ALT and AST levels may not reliably indicate the status of liver inflammation and cirrhosis (61).

A survey of general practitioners (GP) reveals that in normal exercise, there is a sturdy focus on a comparatively small number of selected liver enzymes in the assessment of elevated liver values (62). A Study concludes that serum ALT, AST, and ALP have reveals larger activity in T2D patients with CKD when compared with individuals who do not have CKD (63). Studies reported that mean serum levels of ALP, ALT, and AST were higher and statically significant in the T2D when compared to the non-diabetes(64,65).

Moreover, a significant distinction was reported regarding serum ALT level among DM-CKD group and controls which show that ALT was approximately a 2-fold rise in the adjusted risk of significant steatosis in patients with albuminuria and T2D (66). On the other hand, a study stated that serum ALT is known to reduce with increasing severity of CKD (19). A study showed that ALP was independently related to CKD (20,50). In another research, ALP concentration were higher and statically significantly among individuals with the lowest quartile of UAE in comparing to those in the reduced quartile, and ALP was associated to disturbances of kidney function parameters (42).Studies indicated that total bilirubin levels of patients with diabetic nephropathy were significantly lower than in those without diabetic kidney disease (67,68).

### 2.3. Conceptual Framework

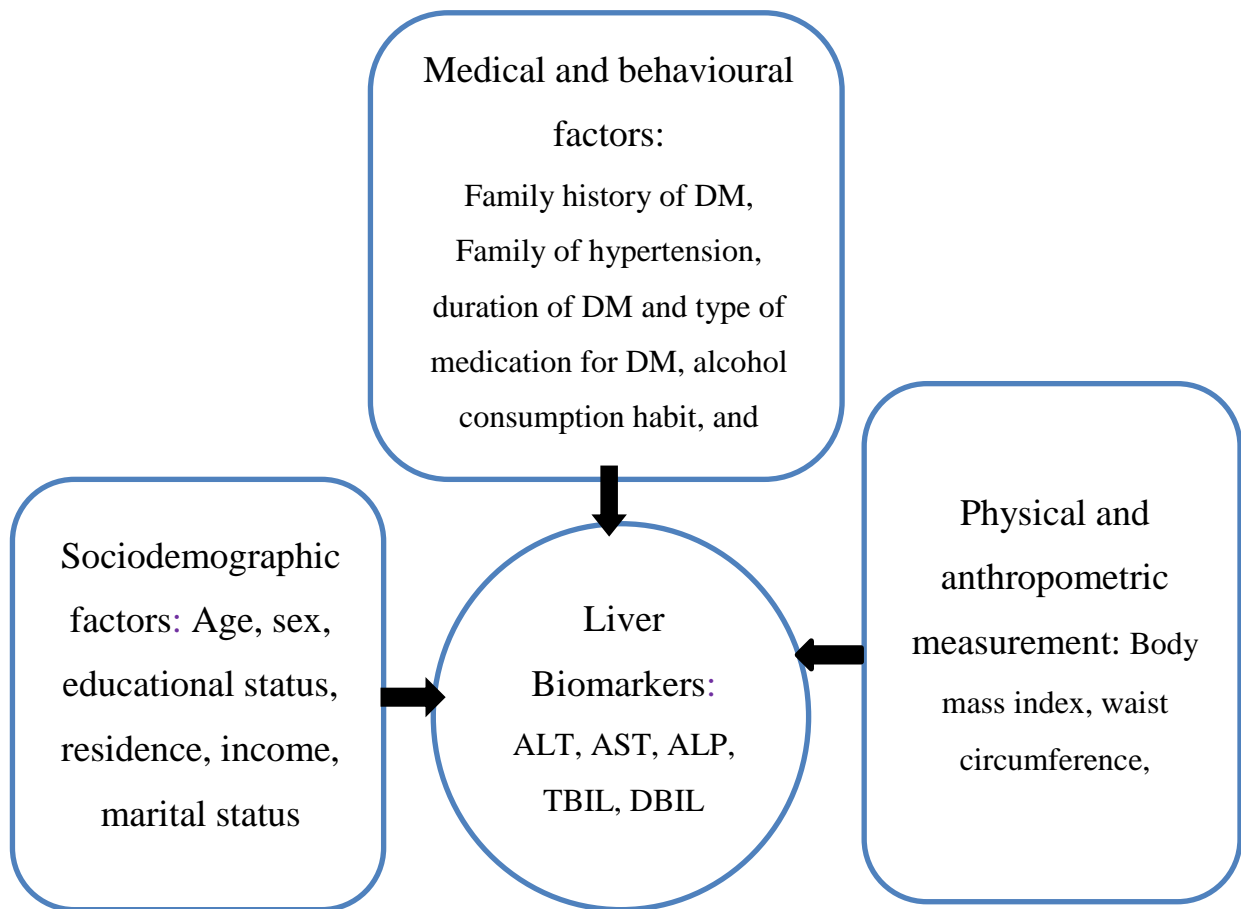


Figure 1: A conceptual framework of Evaluation of liver biomarkers among T2D Patients with or without CKD adapted from various literature with some modification by principal investigator (50,69–71)

## 2.4. Research question

- What is the association between liver biomarkers and T2D patients with CKD?
- What is the association among liver biomarkers and T2D patients without CKD?
- What are associated factors of abnormal liver enzyme in T2D patients?

## 2.5. Hypothesis

### 2.5.1. Null Hypothesis

There is no mean difference of liver biomarker level and its associated factor between T2D patients with CKD group and T2D patients without CKD group.

### 2.5.2. Alternative Hypotheses

There is a mean difference in liver biomarker level and associated factor between T2D patients with CKD group and T2D patients without CKD group.

## 3. Objective of Study

### 3.1. General Objective

The study was aimed to evaluate liver biomarkers and their associated factors among T2D patients with CKD and T2D patients without CKD attending JMC from November 01, 2022 to January 20, 2023.

### 3.2. Specific Objectives

- To assess liver enzymes levels ( ALT, AST and ALP) among T2D patients with and without CKD
- To evaluate TBIL and DBIL among T2D patients with and without CKD
- To identify factors associated with liver biomarkers disorder among T2D patients with and without CKD.

## 4. Methods and Materials

### 4.1. Study Area and Period

The study was conducted at JMC from November 01 to January 20, 2022. The hospital is located in the town of Jimma in southwestern Ethiopia, 354 kilometers away from Addis Ababa, the capital of Ethiopia. The JMC has about 800-bed capacity and serves a total population of approximately 20 million. According to the health management information system and the hospital chronic follow-up registration data, about 600 DM patients were followed up per week in three outpatient chronic follow-ups OPD.

### 4.2. Study Design

An institutional –based comparative cross-sectional study was implemented at JMC.

### 4.3. Populations

#### 4.3.1 Source Population

The source population were all diabetic patients who had Follow up at JMC.

#### 4.3.2. Study Population

Study population were all T2D patients with CKD and T2D patients without CKD and had follow-up at JMC during data collection.

#### 4.3.3. Sample Population

**Group I:** All selected T2D patients with CKD attending JMC during study period and who fulfil inclusion criteria and were recruited during data collection period.

**Group II:** All selected T2D patients without CDK attending JMC during study period and who fulfil inclusion criteria and were recruited during data collection period.

## 4.4. Inclusion and Exclusion Criteria

### 4.4.1. Inclusion Criteria

Adult (age >18 years) T2D patients with and without CKD that are on regular follow-up during a period of data collection and volunteer were included in the study.

### 4.4.2. Exclusion Criteria

Age < 18 years, short follow-up period (<3 months), critically ill patients, patients who have any clinical evidence of any chronic liver disease, any form of bone disease, pregnant and lactating mothers, diabetic patients who are on HIV/TB drugs, other types of DM and patients on hepatotoxic and nephrotoxic drugs was excluded from this study.

## 4.5. Sampling

### 4.5.1. Sample Size Determination

The sample size was calculated by using G power software by using two tailed test to determine mean difference of two independent mean by one-to-one ratio (1:1) and considering the following assumptions:  $\alpha = 5\%$  , power =80% and effect size =0.5. Accordingly, a total of 142 T2D patients, containing 71 T2D patients with CKD and 71 T2D patients without CKD were recruited. Therefore, 71 patients from T2D with CKD and 71 T2D without CKD were incorporated into this study as a sample.

### 4.5.2. Sampling Technique

Each study participant was recruited using consecutive sampling techniques.

## 4.6. Study Variables

### 4.6.1. Dependent Variable

- Liver biomarkers

### 4.6.2. Independent Variables

#### 4.6.2.1. Sociodemographic Factors:-

- Age, sex, marital status, educational status, income,

#### 4.6.2.2. Medical and Behaviour Factors: -

- Duration of DM, type of medication for DM, family history of DM and hypertension  
Smoking status and alcohol consumption habit

#### 4.6.2.3. Physical and Anthropometric Measurements: -

- BMI, waist circumference, weight, measured systolic BP and measured diastolic BP.

### 4.7. Operational Definitions

- **Elevated liver enzymes:** ALT > 45U/L in men;>34U/L in women, AST >35U/L in men ;>31U/L in women, and ALP >129U/L in men and >104 in women (72)
- **Elevated other liver biomarkers:** TBIL>1.2 mg/dl for both sexes, DBIL> 0.2 mg/dl (67).
- **BMI is classified as follows:** underweight  $\leq 18.5 \text{ Kg/m}^2$ , normal weight = 18.5–24.9  $\text{Kg/m}^2$ , overweight =25-29.9  $\text{Kg/m}^2$  and obese  $\geq 30 \text{ Kg/m}^2$  (73).
- **Hypertension:** - if systolic blood pressure  $\geq 140\text{mmHg}$  and diastolic blood pressure  $\geq 90\text{mmHg}$  are considered hypertension, respectively (74).
- **Waist circumference**  $\geq 102/88 \text{ cm}$  in men and women respectively, are obese (75).
- **Group I:** diabetic patients with chronic kidney disease
- **Group II:** - diabetic patients without Chronic kidney disease.
- **Smoking:** Subjects' smoking habits were asked at each single visit. If at least one cigarette per day was reported, patients were classified as smokers(67).
- **Liver biomarkers:** - Include ALT, AST, ALP, TIL ad DIL which are routinely done in our laboratory setup of JMC and in the country as well.

### 4.8. Data Collection and Analysis

#### 4.8.1. Data Collection Instruments

Data were collected using questionnaires, anthropometric measurements, physical examinations and blood samples collecting materials like organ test tube, syringe WC measuring tape BP cuff and glove.

#### 4.8.2 Data Collection

Data were collected using a structured questionnaire after reviewing relevant kinds of literature. The questionnaires were used to collect sociodemographic profiles, behavior-related information, and medical histories of eligible participants. A physical examination was also done, including measurements of height, weight, WC and blood pressure. BMI was calculated using weight and height computed as weight in kilograms divided by height squared in meters. Blood pressure was measured in a standardized fashion two times using the Omron digital BP measuring device (HEM907, Kyoto, Japan) on the right upper arm after participants had been seated and resting quietly for at least five minutes with feet on the ground and back supported. Those patients that take coffee, Coca-Cola, and other substance that increase blood pressure were appointed to come at least after two hours. Consider analyzing the mean of systolic and diastolic blood pressure, as recommended by the WHO. Trained health personnel by introducing himself/herself to respondents collected data and it was collected from those who voluntarily accepted and signed the informed consent form.

#### 4.8.2.1. Blood Sample Collection

A total of 5ml of venous blood was drawn from each volunteer patient using a disposable plastic syringe by professional nurses at the diabetic clinic of JMC. The blood was poured into an organ test tube and centrifuged at 3000rpm for 10 minutes to separate serum from plasma after it clotted then serum was kept at - 80°C until used. On the day of clinical chemistry measurement, a formal laboratory request ordered by physicians was sent to JMC laboratory unit for each selected patient. The results of each study participant were filled in clinical chemistry result report format signed by the sample analyzer and approver. Using an automated machine in the Jimma Medical Center Laboratory automated clinical chemistry analyzer Cobas 6000 core; Tokyo Japan, Roche Diagnostic GmbH, Germany with serial number B801-03) AST, ALT, ALP TBIL, and DBIL were measured according to the manufacturer's procedures.

#### 4.8.3. Laboratory Measurements

##### 4.8.3.1. Alanine Aminotransferase (ALT) Assay Method

ALT the catalysis amino group transfer from alanine to  $\alpha$ -ketoglutarate and leads to glutamate and pyruvate formation. Pyruvate is further react to form lactate by lactate dehydrogenase (LDH) enzyme when NADH exist which is reduction reaction. The chemical reaction is

monitored at 340 nm by a rate of reduce in absorbance due to the oxidation of NADH into NAD<sup>+</sup> via ALT activity that exist in the specimen.

**A) Reagent Composition and Preparation:** Reagent A: lactate dehydrogenase > 1350 U/L, pH 7.3, Tris 150 mmol/L, L-alanine 750 mmol/L, and Reagent B: 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, NADH 1.9 mmol/L, sodium azide 9.5 g/L. Add 4mL of reagent A to 1 mL of reagent B.

**B) Procedure:** Specimens and reagents were mixed up in room temperature and vortexed before analysis. After identifying normal and abnormal high control, 40 specimens at a time were put in a totally automated clinical chemistry analyser. The analyser was calibrated for comfortable temperature, wavelength and volume of specimen in the plasma (50 µl) and 1.0 ml of reagents. The analyser automatically takes enough volume of reagent. Sample and add up then incubates the mixtures at normal body temperature for a minute. Depending on the strength of colour, chemical compound produced, measure the absorbance at 340 nm, at one, two, three minutes and determines the ingredients of ALT in the specimen by calculating the average of the yields to get the change mean in absorbance per minute ( $\Delta A/\text{min}$ ) using Cobas 6000 core; Tokyo Japan, Roche Diagnostic GmbH, Germany with serial number B801-03).

#### 4.8.3.2. Aspartate Aminotransferase (AST) Assay Method

AST catalyses amino group transfer from aspartate to  $\alpha$ -ketoglutarate by the production of glutamate and oxaloacetate. The oxaloacetate was converted into malate by malate dehydrogenase (MDH) in the existence of nicotinamide adenine dinucleotide (NADH) reduced form. The chemical reaction was monitored at 340 nm by the rate of reduce in absorbance due to the oxidation from NADH to NAD<sup>+</sup> proportional to the catalytic activity of AST exist in the specimen.

**A) Reagent Composition and Preparation:** Reagent A, L-aspartate 362 mmol/L, Tris 121 mmol/L, lactate dehydrogenase > 660 U/L, malate dehydrogenase > 460 U/L, sodium hydroxide 255 mmol/L, pH 7.8, Reagent B: 2-oxoglutarate 75 mmol/L, NADH 1.9 mmol/L, sodium azide 9.5 g/L. Mix 4 mL, sodium hydroxide 148 mmol/L. mix 4mL of reagent A to 1 mL of reagent B

B) Procedure: specimens and reagents were mixed at room temperature and vortexed before analysis. After adding normal and abnormal high control, 40 specimens at one time were put in a totally automated clinical chemistry analyser. The analyser was calibrated for comfortable temperature, wavelength and volume of plasma specimen (50 µl) and 1.0 ml of reagents. The analyser automatically takes enough volume of reagent and specimen, mixes together and incubates the mixtures at normal body temperature for one minute. Depending on the strength of colour compound formed, the automated analyser machine automatically reads the absorbance at 340 nm at one, two, three minutes and determines the ingredients (activity) of AST in the specimen by calculating the average of the results to gain the mean alteration in absorbance per minute ( $\Delta A/\text{min}$ ).

#### 4.8.3.3. Alkaline Phosphates Assay Method

Alkaline phosphatase degrades *p*-nitrophenyl phosphate and this phosphate would be transferred to AMP (2-Amino-2-methyl-1-propanol.). The raise in absorbance at 405 nm at 37°C was reads and this could be proportional to the concentration of alkaline phosphatase that is available in the specimens. The method is linear up to 1200 U/L.

A) Reagent ingredient and Preparation: Reagent 1: Buffer 2-Amino-2-methyl-1-propanol (AMP) and magnesium acetate and Reagent 2: Substrate: *p*-nitrophenyl phosphate 60 mmol/L, sodium azide 0.095 %. Two mL of reagent2 will be added to one bottle of reagent 1 and mixed strongly prior to utilize.

B) Procedure: After adding specimens and chemical reagents to the optimum room temperature, 20µl of plasma was brought together with 1.0 ml of reagent and incubated at normal body (37°C) for one minute. The starting absorbance was measured and followed exactly after one, two and three minutes.

#### 4.8.3.4. Total Bilirubin Determination Method

Bilirubin is changed into coloured compound known as azobilirubin by diazotized sulfanilic acid and its absorbance is determined spectrophotometrically at 540 nm. The strength of colour produced is exactly proportional to the amount of bilirubin in the sample. Out of direct and bilirubin, small in plasma in plasma (conjugated and unconjugated) only the conjugated elicits chemical reaction directly, whereas free bilirubin elicits chemical reactions after being

removed from albumin by an accelerator compound. The procedural method is linear up to 20 mg/dl. Total bilirubin + Sodium Nitrite+ Diazotized Sulfanilic Acid = Azobilirubin

Reagent Composition and Preparation: R1: Sulfanilic acid 29 mmol/L, HCL 0.24 mol/L, duposol 3% (w/v); R2: Sodium nitrite 11.6 mmol/L. Mix 1 ml RN + 4 ml RT.

Procedure: Specimens and chemical reagents were mixed to optimum temperature of room and every plasma was vortexed into every of labelled respective tubes and the reagents will be added by pipette. The reaction mixture was brought and permitted to stay at optimum temperature for 2 minutes. The absorbance of specimens and standard was measured toward reagent blank at 540 nm using Cobas 6000 core automated clinical chemistry analyser. Calculation: The absorbance change of standard and samples was determined as follows:

$$\Delta A \text{ sample} = A \text{ sample} - A \text{ blank}$$

$$\Delta A \text{ standard} = A \text{ standard} - A \text{ blank}$$

Sample total bilirubin concentration will be calculated using the following equation:

$$\text{Sample T. Bilirubin Concentration (mg/dL)} = \frac{\Delta A \text{ sample} \times (\text{Concentration of Standard})}{\Delta A \text{ standard}}$$

$$\Delta A \text{ standard}$$

#### 4.8.4. Data Quality Control Measures

The data quality begins with the sample collection. The questionnaire were prepared in English and translated into Amharic and Afan Oromo then back to English. The accuracy of the questionnaires was tested by conducting pre-test on 5% of the actual sample size who fulfilled the criteria at Shanan Gibe Hospital. Based on the finding of the pre-test, the questionnaires were revised. The averages of two measurements were used to determine blood pressure values. The sample had been collected by aseptic techniques and considering the proper procedure. Trained health professionals collected the data after one day of training was given to the data collectors on the specifics of the project data. Every day the principal investigator checked the collected data and any incomplete documents were cleaned and checked for quality before data entry.

#### 4.8.5. Data processing and Analysis

After collected the data were checked for completeness and cleanness. Up on completion, EpiData version 4.6.0.6 software was used for data entry and then was exported to statical package for social science (SPSS) version 26 software for analysis. Assumption like normality, homogeneity, linearity, multicollinearity and outliers were checked and met using Kolmogorov-Shapiro Wilki's test, leaven's statics, scatter plot, tolerance value and VIF and cooks distance respectively. All continuous and normally distributed data were expressed as mean  $\pm$  standard deviation and categorical data was expressed as proportions or percentages. Pearson correlation was calculated to see the correlation between liver biomarkers levels with various factors. Bivariate and multivariate logistic regression models were performed to test the significance of the association. Data was compared using two independent t-tests to test the significance of mean differences between groups. A variable associated at p-value  $< 0.05$  was considered statistically significant.

#### 4.9. Ethical Consideration

Ethical clearance and supportive letter was obtained from the Research and Ethical Committee of the Jimma University Institute of Health Sciences Ethical Review Board with number of JUIH/IRB/242/2. A structured questionnaire (attached as an appendix) and consent form was prepared with a detailed explanation of the objectives, risks, and benefits of the study and the confidentiality of responses was maintained. Data was collected after obtaining informed consent and agreement from the patients under study. Trained health professionals (BSc nurse) performed sample collection as, the ethical steps and scientific procedures

#### 4.10. Dissemination Plan

At the end of the study, the result will be submitted to Jimma University, Institute of Health, Faculty of Medical Sciences, and Department of Biomedical Sciences. It will also be presented in seminars and other scientific meetings as well as disseminated to all concerned bodies and effort will be made to publish the data in the data in this thesis in peer-reviewed international academic journals.

## 6. Results

### 6.1. Sociodemographic Characteristics of Study Population

Of the total the study participants, 75(52.8%) were males. Among these 73(51.4) are Muslim, 47 (33.1) are Orthodox Christian and 22 (15.5) are protestant religion followers. In addition, based on their educational status the majority of study participants was primary School 53 (37.3), followed by Secondary School and above 36 (25.4). Also 34 (23.9) can read and write but 19 (13.4) people were illiterate.

Table1: General description of Sociodemographic factor of the participants

Variable		Number	Percent (%)
Age	<35 years	7	4.9
	36- 50 years	52	36.6
	51-65 years	53	37.3
	>=65	30	21.1
Sex	Male	75	52.8
	Female	67	47.2
Religion	Protestant	22	15.5
	Orthodox	47	33.1
	Muslim	73	51.4
Educational status	Illiterate	19	13.4
	Can read and write	34	23.9
	Primary school(1-8)	53	37.3
	Secondary and above	36	25.4
Marital status	Single	10	7
	Married	112	78.9
	Divorced	6	4.2
	Widowed	14	9.9
Occupation	Government employee	23	16.2
	Housewife	27	19.0
	Farmer	50	35.2
	Student	5	3.5
	Merchant	23	16.2
	Daily laborer	11	7.7
	Others	3	2.1
Average monthly income	<1000 ETB	34	23.9
	1001 -3000 ETB	65	45.8
	3001 -5000 ETB	23	16.2
	>5000 ETB	20	14.1

## 6.2. Medical and Behavioural Factors

As in table2, among the participant majority, 104 (73.2%) have family history of both DM and hypertension and the rest 38 (26.8%) have no family history of both DM and hypertension. Additionally, types of medication taken for DM were assessed and majority 43 (30.3%) take Insulin plus metformin, and small number 19(13.4%) of the participants took Glabineclamaide only. On the other hand, around 118(83.1) subjects were non-smoker and 24(16.9) were smoker.

*Table 2: General description of medical and behavioural factors*

Variables		Number	Percent
History of HTN	Yes	38	26.8
	No	104	73.2
Family history of DM	Yes	104	73.2
	No	38	26.8
Duration of diabetes	<5 years	24	16.9
	6 - 10 years	58	40.8
	11-15 years	44	31.0
	>15 years	16	11.3
Types of the medication for DM	Insulin only	28	19.7
	Metformin only	31	21.8
	Glabineclamaide only	19	13.4
	Insulin +Metformin	43	30.3
	Metformin and Glabineclamaide	21	14.8
Smoking	Yes	24	16.9
	No	118	83.1
Alcohol consumption	Yes	20	14.1
	No	122	85.9

## 6.3. Physical and Anthropometric Factors

As revealed in table3: the mean value of height of study subject was  $1.69 \pm 0.09$  in meter and an average weight was about  $70.46 \pm 10.06$  in kilogram. Majority of participant, 76 (53.5), 58 (40.8) and 8 (5.6) have normal, overweight, and obese in calculated BMI respectively. On other hand large number (66.9%) of subjects have normal waist circumference.

Table 3. Description of physical and anthropometric measurement

Variables		Number (n) or mean±SD	Percent (%)
Height(m)	(Mean±SD)	1.69 ±.09	
Weight (kg)	(Mean±SD)	70.46 ±10.06	
BMI(kg/m <sup>2</sup> )	18.5 - 24.9,normal	76	53.5
	25 -29.9,overweight	58	40.8
	>=30 obese	8	5.6
WC(cm)	Normal	95	66.9
	Higher/ Obese/	47	33.1
ASBP(mmHg)	Normal	53	37.3
	High	89	62.7
ADBP(mmHg)	Normal	49	34.5
	High	93	65.5

Data were mean for weight and height number and percentage height and weight and number for others. Kg= kilogram, mmHg = millimetre of mercury, cm= centimetre

#### 6.4. Comparison between DM with CKD and DM without CKD

##### 6.4.1 Comparison in Terms of Sociodemographic

As shown in Table 4, 5 and 6 although, some of this data does not necessarily imply a correlation between the disease situation and the habit, proportions (percentage) was compared between group 1 and group 2 to know characteristics of study subjects in the group. Majority 49 (69%) of the T2D patient with CKD were older than 50 years when compared with T2D without 29 (40.8%). About 35 of group 1 were female and 32 of group 2 are female. Most of the study participants are Muslim religion followers, followed by Orthodox Christian and Protestant Christian holds small number in both group 1 and group 2.

Table 4: Comparison in terms of Sociodemographic factors, Jimma University, southwest, Ethiopia, 2023.

Variables		Study participants		
		DM with CKD	DM without CKD	Total Sample
Age (years)	=<35	1 (1.4)	9 (12.3)	10 (7)
	36 -50	21 (29.6)	33 (46.5)	54 (38)
	51-65	29(40.8)	20 (28.2)	49 (34.5)
	>65	20 (28.2)	9 (12.3)	29 (20.5)
Sex	Male	36 (50.7)	39 (54.9)	75 (52.8)
	Female	35 (49.3)	32 (45.1)	67 (47.2)
Religion	Protestant	14 (19.7)	8 (11.3)	22 (15.5)
	Orthodox	23 (32.4)	24 (33.8)	47 (33.1)
	Muslim	34 (47.9)	39 (54.9)	73 (51.4)
Educational status	Illiterate	11 (15.5)	8 (11.3)	19 (13.4)
	Can read & write	19 (26.8)	15 (21.1)	34 (23.9)
	Primary	23 (32.4)	30 (42.3)	53 (37.3)
	Secondary & above	18 (25.4)	18 (25.4)	36 (25.4)
Marital status	Single	1 (1.4)	9 (12.7)	10 (7)
	Married	61 (85.9)	51 (71.8)	112 (78.9)
	Divorced	0(0)	6 (8.5)	6 (4.2)
	Widowed	9 (12.7)	5 (7)	14 (9.9)
Occupation	Government employee	17(23.9)	6(8.5)	23(16.2)
	Housewife	10(14.1)	17(23.9)	27(19)
	Farmer	27(38)	23(32.4)	50(35.2)
	Merchant	12(16.9)	11(15.5)	23(16.2)
	Others*	5(7.1)	14(19.7)	19(13.4)
Average monthly income	<1000 ETB	15(21.1)	19(26.8)	34(23.9)
	1001 -3000 ETB	33(46.5)	32(45.1)	65(45.8)
	3001 -5000 ETB	10(14.1)	13(18.3)	23(16.2)
	>5000 ETB	13(18.3)	7(9.9)	20(14.1)

#### 6.4.2. Comparison in Terms of Medical and Behaviour Factors

DM patients with CKD have longer duration of DM When compared with group II. About 51 (71.8%) of group I have family of DM which is less than that of group II (74.6%). Small numbers of group I (26.8%) have family history of hypertension that is larger when compared with group II 23.9%. Larger numbers of group I (18.3%) were smoker when compared with T2D without CKD (11.3%). Similarly, eleven of the group I has alcohol consumption history while eight of GROUP II have alcohol consumption. Finally, 36.6% of T2D patients with CKD take insulin plus metformin where as 23.5% in case of group II take it.

Table 5: Comparison in terms of medical and behaviour factors, Jimma University, Jimma, Ethiopia ,2023 (n=142)

Variables		Study Subjects		
		DM with CKD	DM without CKD	Total sample
History of HTN	Yes	19 (26.8)	17 (23.9)	36 (25.4)
	No	52 (73.2)	54 (76.1)	106 (74.6)
History of DM	Yes	51 (71.8)	53 (74.6)	104 (73.2)
	No	20 (28.2)	18 (25.4)	38 (26.8)
Duration of DM	<5years	0 (0)	24(33.8)	24 (16.9)
	6-10 years	31(43.7)	27 (38)	58 (40.8)
	11-15 years	26 (36.6)	18 (25.4)	44 (31.0)
	>15 years	14(19.7)	2 (2.8)	16 (11.3)
Types of medication used for DM	Insulin only	6(8.5)	22(31)	28 (19.7)
	metformin only	14(19.7)	17(23.9)	31 (21.8)
	Glabineclamaide only	11(15.5)	8(11.3)	19 (6.3)
	Insulin +metformin	26(36.6)	17(23.9)	43 (30.3)
	metformin and Gc	14(19.7)	7(9.9)	21 (14.8)
Smoking	Yes	13 (18.3)	8 (11.3)	21 (14.8)
	No	58 (81.6)	63 (88.7)	121 (85.2)
Alcohol consumption	Yes	11 (15.5)	8 (12.7)	20 (14.1)
	No	60(84.5)	62 (87.3)	122 (85.9)

Data were numbers and Percentages. HTN= hypertension, Gc = Glabineclamaide.

#### 6.4.3. Comparison in Terms of Physical and Anthropometric Measurement

As in the table 6: T2D patients with CKD have less mean value weight ( $68.2\pm 1.1$ ) when compared with group II ( $72.7\pm 1.3$ ). On the other hand, DM-CKD patients have higher height ( $1.7\pm 0.01$ ) when compared with group II ( $1.68\pm 0.01$ ). Small number, (BMI=29.6%, WC= 32.4%) of DM patients with CKD had larger BMI and WC when compared with DM patients without CKD. On the other hand, larger numbers of group I have higher both systolic and diastolic blood pressures when compared with group II (ADBP=42.3%, ASBP=38%).

Table 6: Comparison of group I and group II population in terms of physical and anthropometric measurement, Jimma University, Ethiopia, 2023 (n=142)

Variables		Study Subjects		Total sample
		DM with CKD	DM Without CKD	
Weight(kg)	Mean $\pm$ SD	68.20 $\pm$ 1.05	72.72 $\pm$ 1.27	70.46 $\pm$ 10.06
Height (m)	Mean $\pm$ SD	1.70 $\pm$ 0.01	1.6759 $\pm$ 0.01	1.69 $\pm$ .09
BMI(kg/m <sup>2</sup> )	18.5 - 24.9,normal	50(70.4)	26 (36.6)	76(53.5)
	25 -29.9,overweight	19(26.8)	39 (54.9)	58 (40.8)
	$\geq$ 30 obese	2(2.8)	6 (8.5)	8 (5.7)
WC(m)	Normal <102/88 M &F	48(67.6)	47(66.2)	95(66.9)
	Higher/obese $\geq$ 102/88	23(32.4)	24(33.8)	65(45.8)
ASBP (mmHg)	normal=BP<140mmHg	44(62)	45(63.4)	89 (62.7)
	High =BP $\geq$ 140mmHg	27(38)	26 (36.6)	53(37.3)
ADBP (mmHg)	normal =DBP <90mmHg	41 (57.7)	52 (73.2)	93 (65.5)
	High = DBP $\geq$ 90mmHg	30 (42.3)	19 (26.8)	49 (34.5)

Data were mean  $\pm$ SD for weight and height but number and Percent for others, mmHg= millimetre of mercury, kg= kilogram and m= meter

#### 6.2.4. Mean Comparison among T2DM with CKD and without CKD Participants

An independent samples t-test was performed to compare means of liver biomarkers and associated factors among T2D patients with CKD and without CKD participants. Assumptions of independent samples t- test (normality and homogeneity) were performed and met. Based on findings of Kolmogorov-Shapiro-Wilk and Levine's tests when  $p > 0.05$ .

T2D patients with CKD had significant mean value of older age and longer duration of DM (Mean  $\pm$ SD=56.87  $\pm$ 10.64 and 11.80 $\pm$ 3.77 respectively) when compared to group II (mean  $\pm$  SD = 48.69  $\pm$  11.53 and 7.91  $\pm$  3.93). Additionally, the mean values of both systolic and diastolic blood pressure, liver biomarkers (ALP, TBIL and DBIL) were significantly higher in T2D Patients with CKD than T2D without CKD. AST and ALT are larger but statically not significant. In contrast, weight, BMI and WC were significantly lower (mean  $\pm$ SD of BMI= 23.76  $\pm$  3.13 and WC=83.34  $\pm$  19.53) when compared to T2D patients without CKD (mean  $\pm$ SD of BMI=25.91  $\pm$  3.03 and WC= 89.50  $\pm$ 14.70) at the  $p < 0.05$  by using independent t- test analysis.

Table 7: Mean Comparison of Sociodemographic, Clinical and Anthropometric Characteristic of Study Participants Jimma University, Southwest, Ethiopia, 2023

Variables	Study subjects			P-value
	Total Sample	T2D with CKD	T2D without CKD	
Age	52.78±11.79	56.87 ±10.64	48.69 ± 11.53	<0.001
DD(years)	9.87±4.73	11.80±3.77	7.91 ± 3.93	<0.001
Weight(kg)	70.44±10.09	68.20 ±8.88	72.71 ±10.76	0.008
BMI(kg/m <sup>2</sup> )	24.30±0.702	23.76 ± 3.13	25.91 ± 3.03	0.001
WC(cm)	87.42 ±17.51	83.34 ± 19.53	89.50 ±14.70	0.035
ASBP (mmHg)	131.39± 13.54	134.20 ±13.19	128.59 ±13.39	0.013
ADBP (mmHg)	81.06±9.26	84.13 ± 8.27	78.46 ± 9.90	<0.001
AST(U/L)	27.32±10.76	28.25 ±10.83	26.39 ±10.70	0.305
ALT(U/L)	32.78±13.98	34.38 ±14.44	31.17 ± 13.40	0.172
ALP(U/L)	106.35 ±27.62	115.27 ± 22.41	97.42 ± 29.57	<0.001
TBIL(mg/dl)	0.94±0.47	0.86 ± 0.47	1.03 ± 0.48	0.028
DBIL(mg/dl)	0.185±0.083	0.16 ± 0.083	0.21 ±0.09	0.003

Data were mean ±SD, mmHg = millimetre of mercury, mg/dl = milligram per decilitre, U/L = units per litter cm= centimetre, kg = kilogram, kg/m<sup>2</sup> = kilogram per meter square

### 6.5. Correlation Analysis of Independent and Dependant Variables

Factors which may affect the liver biomarkers were including age, BMI, diabetic duration (DD) ASBP, ADBP and WC. However, these factors have no significant strong correlation with most of liver biomarkers. Nevertheless, there is significant moderate negative correlation between ALP and age ( $r = -0.128$ ,  $p < 0.031$  (table 8)). We also observe a weak positive correlation between ALT and DD ( $r = 0.168$ ,  $P < 0.045$  and positive correlation between DBIL and WC ( $r = 0.243^{**}$ ,  $p = 0.004$ ).

Table 8: Bivariate correlation among continuous variables in the study subjects Jimma, Ethiopia 2023

Independent Variables	Pearson coefficient(r)	Outcome variables				
		ALP	ALT	AST	DBIL	TBIL
Age (year)	R	-0.128*	0.099	0.060	0.28	-0.28
DD (years)	R	-0.013	0.168*	0.044	0.058	-0.003
Weight (kg)	R	0.022	0.126	-0.043	0.088	-0.020
ASBP (mmHg)	R	0.086	-0.021	0.019	0.008	-0.024
BMI (kg/m <sup>2</sup> )	R	0.043	-0.087	-0.041	-0.079	-0.145
WC (cm)	R	-0.152	0.110	-0.032	0.243**	0.113

\*and \*\*correlation were significant at  $p < 0.01$  and  $0.05$  respectively.  $r =$  Pearson correlation coefficients, DD= diabetic duration, ASBP=average systolic blood pressure, BMI=body mass index

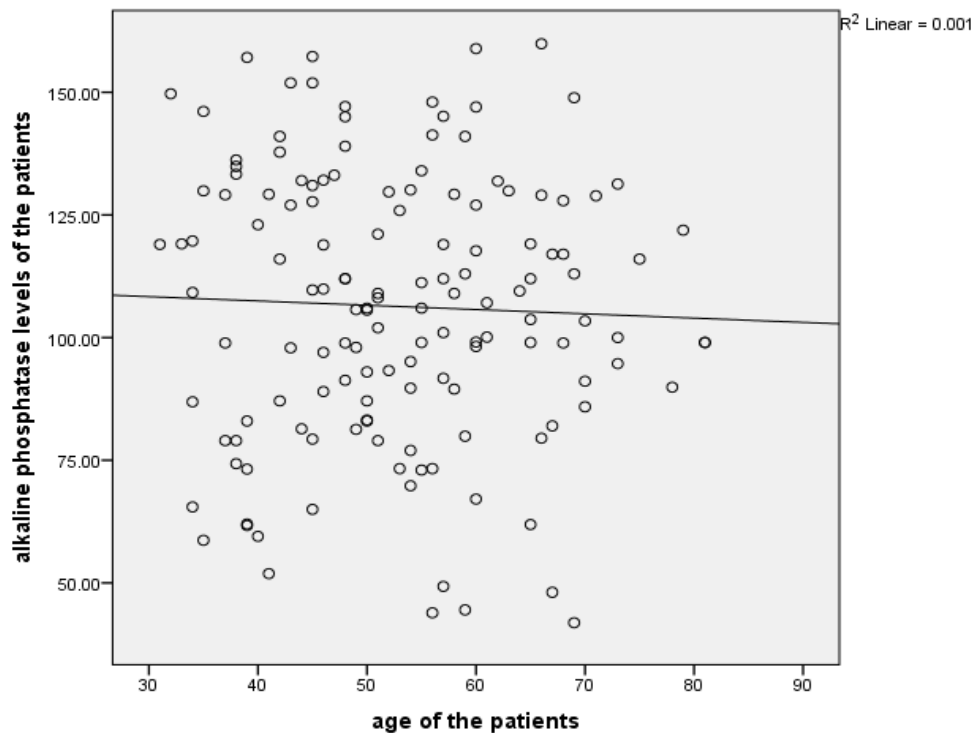


Figure 2:-Scatter plot showing negative correlation between age of the patients and ALP level

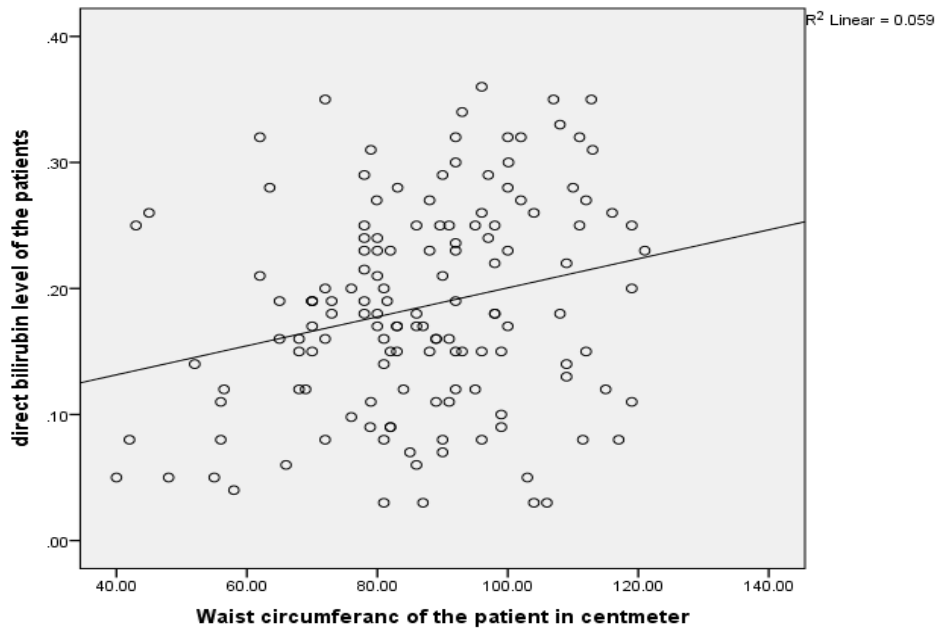


Figure 3:-Scatter plot showing positive correlation between WC and DBIL level

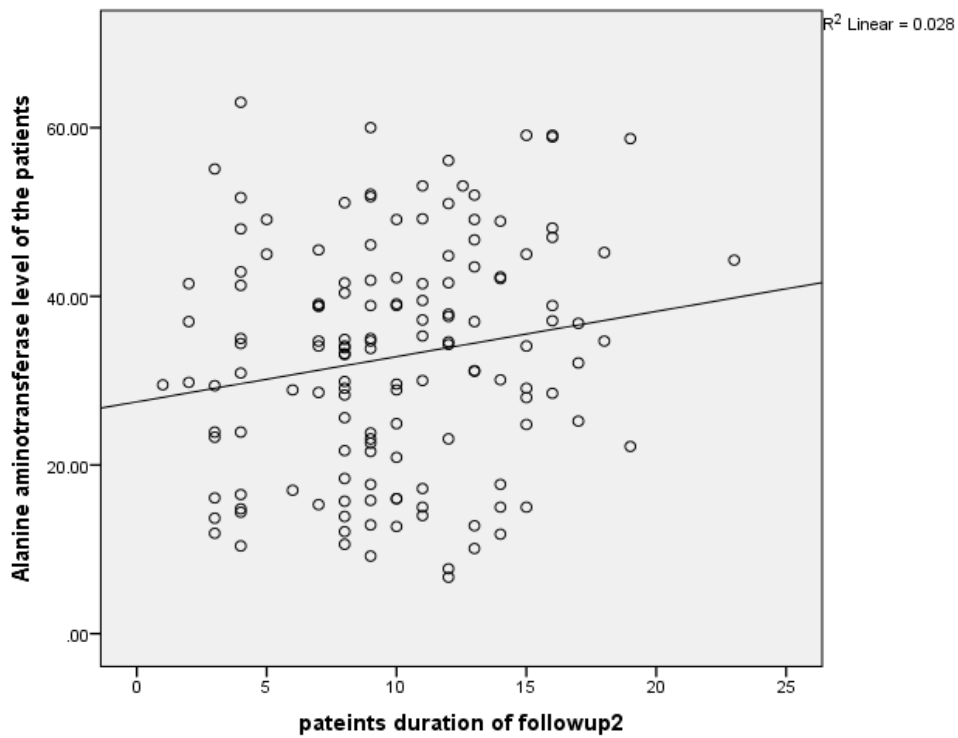


Figure 4:-Scatter plot showing positive correlation between duration of DM and ALT level

## 6.6. Bivariate and Multivariate Logistic Regression Analysis

Since there is no significant variable in simple linear regression, identifying associated factors by logistic linear regression. So, after adjustment for cofounding variable, a bivariate and multivariate logistic regression was analysed to predict participants' AST, ALT, ALP, TBIL and DBIL based upon different variables. The assumptions of logistic regression (normality, homogeneity, outlier, and linearity) was checked and met. There was significant association between the AST and factors like smoking history, ASBP and ADBP; ALT and predictors like smoking, DD and ASBP, ALP with presence of CKD. Additionally DBIL and TBIL were associated with presence of CKD.

Table 9: Bivariate and Multivariate logistic regression analysis of factors associated with liver biomarkers

Variable	Category	AST		COR	AOR	P-value
		Elevated	Normal			
Smoking	No	29(25.6)	89(74.4)	1	1	0.008
	Yes	13(52.4)	11(47.6)	3.6(1.45,8.97)	3.8(1.42,10.01)	
ASBP(mm Hg)	Normal	9(17)	44(83)	1	1	0.035
	High	33(37.1)	56(62.9)	2.8(1.20,6.39)	2.6(1.07,6.14)	
ADBP (mmHg)	Normal	9(18.4)	40(81.6)	1	1	0.033
	High	33(35.5)	60(64.5)	2.4(1.057,5.66)	2.7(1.08,6.60)	
Variable	Categories	ALT Level		COR(95%CI)	AOR (95%CI)	p-value
		Elevated	Normal			
Smoking	No	49(41.5)	69(58.5)	1	1	0.04
	Yes	17(70.8)	7(26.2)	3.4(1.32,8.87)	2.9(1.05, 7.78)	
Duration of DM	<5 years	8(33.3)	16(66.7)	1	1	0.027
	>15 years	11(68.7)	5(31.3)	4.4(1.13,17.10)	5.2(1.20,22.50)	
ASBP (mmHg)	Normal	15(28.3)	38 (71.7)		1	0.001
	High	51(57.3)	38(42.7)	3.4(1.64,7.06)	3.8(1.74,8.50)	
Variable	Categories	ALP level		COR(95%CI)	AOR(95%CI)	P-Value
		Elevated	Normal			
Dx group	DM	20(28.2)	51(71.8)	1	1	0.029
	DM-CKD	33(46.5)	38(53.5)	2.21(1.10,4.44)	2.2(1.09, 4.46)	
Variables	Categories	DBIL		COR(95%CI)	AOR(95%CI)	P-value
		Elevated	Normal			
Dx group	DM-CKD	17(23.9)	54(76.1)	1	1	0.009
	DM	31(43.7)	40(56.3)	2.5(1.20,5.05)	2.8 (1.29,6.06)	
Variable	Categories	TBIL		COR(95%CI)	AOR(95%CI)	P-value
		Elevated	Normal			
Dx group	DM-CKD	19(26.8)	52(73.2)		1	<0.0001
	DM	45(63.4)	26(36.6)	4.7(2.32,9.67)	5.14(2.24,11.56)	

AOR=adjusted odds ratio, CI = confidence interval COR=crudes odds ratio,



## 7. Discussion

Diabetes mellitus (DM) is the leading cause of chronic kidney disease (CKD) which in turn causes macrovascular and microvascular (22). T2D is considered to be a main cause for ESRD, lower-extremity ulcer that leads to amputations, and adulthood visual impairment (2). Liver function test is useful for evaluation and treatment of patients with hepatic disorder among T2D patient with CKD (48). To the best of our knowledge, this is the first report in measuring and comparing liver biomarkers among T2D patient with CKD and T2D patients without CKD in Ethiopian population.

As shown in the Table 7 a mean value of ALP level was larger in T2D patient with CKD when compared with T2D patients without CKD ( $P < 0.001$ ). This report is supported by other study (20,76). Mechanisms of this difference may be cell membrane destruction at large ingredients, mitochondrial malfunction, toxin substance production, and main metabolism steps regulation distortion. Other explanations could include oxidative stress and gathered inflammatory marker cells which responsible for increased pro-inflammatory cytokines like tumour necrosis factor- $\alpha$  (TNF-  $\alpha$ ), that may leads to hepato-cellular damage (64). ALP is capable of affecting inorganic phosphate (Pi): pyrophosphate (PPi) ratio that plays crucial role in developing vascular calcification (77). Other mechanisms related ALP to endothelial malfunction could be decreasing of tyrosine kinase action in the endothelial cells which result in defect of endothelial nitric oxide (NO) synthase function, help for high formation of ROS, and programmed cell death due to elevated breakdown of pyrophosphate which increase atherosclerotic injury in vascular wall (78).

However, the mean value of other liver biomarkers like TBIL and DBIL were decreased and statically significant among T2D patients who have CKD comparing with T2D without CKD ( $p = 0.028$  and  $p = 0.003$ ) respectively. These results is in agreement with other studies, which indicated that TBIL levels of patients who have diabetic kidney disease were lower than in those without diabetic nephropathy (67,68). This could be due to that bilirubin has capacity of binds to albumin in blood plasma and is sent into the liver, and the ingredient of unconjugated plasma bilirubin is very small (79). Mechanisms for this may include, Bilirubin has an antioxidant activities which has been investigated to inactivate lipid oxidation, the immune response, inflammation, proliferation of cell and programmed cell death (80).

On average, T2D with CKD are significantly older when compared with DM patients without nephropathy ( $p < 0.001$ ). This finding is consistent with others studies, which reveals that older age was known to be the strong predictor of reduced eGFR and has been statically associated with elevated incidence of nephropathy in diabetic patients (4,51,59). This may be because of aging leads to significant decrease in kidney function by promoting chronic inflammation which contribute to fibrosis and age related condition that decline organ function. Additionally, ageing is known to associated to a proinflammatory condition that manifested as large levels of proinflammatory markers, a condition called “inflammageing” which is strongest risk factor for kidney diseases (4,51,59,77). The mean value of diabetic duration of follow up is significantly higher among T2D patients with CKD in comparison with T2D without CKD ( $p < 0.001$ ). The current result were supported by others studies done in others area (40,48). Possible pathogenesis of long term persistent hyperglycaemia has been associated with functional and structural changes in both liver and renal cells (41).

On the other hand, DM patients with CKD have significantly lower mean weight, BMI and WC than those without CKD ( $p=0.008$ ,  $p=0.001$  and  $p = 0.035$ ) respectively. This result is supported by others studies (40,52,59,81). These lowering of mean value, BMI and WC among subjects with CKD observed in this study could be because of renal failure that linked to weight loss (82) which in turn related to loss of appetite and metabolic Acidosis that would reduce both muscle and fat mass specially in ESRD (19). However, the mean value of WC was completely contraindicated with what was reported by AL-Ghonim et al in Saudi-Arabia (40). This discrepancy may be because of sample size difference in this case or life style of the study participant and might be due to the difference in population in terms of geographical location and racial background.

Additionally, the mean value of systolic blood and diastolic blood pressure were found to be higher in T2D patients with CKD in comparison T2D patients without CKD ( $p = 0.013$  and  $p < 0.001$ ) respectively. This finding is supported with other studies which reported that elevated systolic blood pressure ( $\geq 140$ mmHg) was associated with a higher prevalence of CKD including diabetic nephropathy (30,51,52). The mechanisms might be because of activation of sympathetic nervous system, endothelial cell dysfunction, renin-angiotensin aldosterone system, excess sodium retention and oxidative stress. Elevated BP resulting in elevated intra glomerular pressure, affecting the glomerular filtration rate that leading CKD (2,83).

As it is in table 8, the results have focused on factors that may assume to affect the dependent variables (Liver biomarkers level) like age, BMI, WC, Diabetic Duration, SBP and DBP. However, these factors had no significant strong correlation with most of the LFTs. Nevertheless, there was significant weak negative correlation between: ALP and age were negatively correlated. Additionally, duration of diabetics follow up (DD) and Alanine aminotransferase (ALT) levels have very weak positive correlation. Waist circumference (WC) and direct bilirubin levels have also weak positive correlation. These findings are consistent with other study (31). In this research, TBIL has moderately positive relationship with WC but not significant. This discrepancy may be due to small sample size in this study and patient stage of renal disease.

Bivariate and Multivariate logistic analysis were performed to assess whether selected independent variables like age, sex, weight, BMI, WC, Measured blood pressure, presence of CKD, DM duration, and types of medication, history of hypertension, cigarette smoking, alcohol intake and family history of DM were predict and liver biomarkers test (AST, ALT, ALP, TBIL and DBIL) disorders have effect on outcome variables. Accordingly smoker patients were 2.9 and 3.8 times more likely to have elevated ALT and AST than those who were not smoking (AOR=2.9 (1.05, 7.78),  $p = 0.008$  and 3.8 (1.42, 10.01),  $p = 0.04$ ) respectively. These findings are in line with other research which states that smoker were like to have elevated ALT (55,56). This might be because of smoking build up fat in the liver that leads to possible progression into serious liver disease which is associated to nicotine; where it cause significant increase the mean of AST,ALT and ALP (84).

ASBP is also significantly associated with elevated AST and ALT ( $p = 0.035$  and  $= 0.001$ ) respectively. This finding was in agreement with other research which indicated that high systolic and diastolic blood pressure has 2.16 and 1.68 times risk of elevated ALT and AST respectively (48,55). Possible pathophysiology of this may be aminotransferases associated with higher dyslipidaemia which leads to elevated serum TC and high LDL, low HDL cholesterol and higher ALT levels are associated with excessive inflammations and excess oxidative stress (55). Participants whose diabetic duration were  $>15$  years were more likely to have 5.2 times elevated ALT level ( $p = 0.027$ ). Possible pathogenesis of long term persistent hyperglycaemia has been associated with functional and structural changes in cells due to metabolic disturbance like metabolic acidosis, reactive oxygen species production, generation of glycation end product and activation of different signalling pathways that leads to leakage

of aminotransferase (55). Diabetic Patients with CKD were 2.2 times more likely to have elevated ALP levels when compared with DM- patients without CKD ( $p= 0.028$ ). This result is in line with other study which revealed that serum ALP level Patients with serum ALP  $\geq 97$  IU/L had a 13.8 higher risk for ESRD (78). Possible mechanisms may be cell membrane destruction at large ingredients, mitochondrial malfunction, toxin substance production, and main metabolism steps regulation distortion. Other explanations could include oxidative stress and gathered inflammatory marker cells which responsible for increased pro-inflammatory cytokines like tumour necrosis factor- $\alpha$  (TNF-  $\alpha$ ), that may leads to hepato-cellular damage (64). ALP is capable of affecting inorganic phosphate (Pi): pyrophosphate (PPi) ratio that plays crucial role in developing vascular calcification (77). Other mechanisms related ALP to endothelial malfunction could be decreasing of tyrosine kinase action in the endothelial cells which result in defect of endothelial nitric oxide (NO) synthase function, help for high formation of ROS, and programmed cell death due to elevated breakdown of pyrophosphate which increase atherosclerotic injury in vascular wall (76,78). In contrary, DM patients without CKD were 2.8 and 5.1 times more likely to have elevated both DBIL and TBIL than T2D with CKD ( $P=0.009$  and  $P< 0.001$ ) respectively. This mean, it was observed that both patients with reduced serum total and direct bilirubin were significantly associated with risk of diabetic kidney disease. This is supported by others findings with cohort studies which stated that DKD less likely to have risk of elevated TBIL (85,86). The possible mechanisms for association between TBIL and DKD; long term high blood sugar can results in elevated of mitochondrial reactive oxygen species (ROS) from endothelial cells, which is major cause vascular injury, and result the basic injury to the kidney (85).

**Strength and Limitation:** The strength of this study was providing some sorts of information on the association routine liver biomarkers among DKD individuals. In addition, this study is the first to measured liver biomarkers level T2D patients with CKD in population of Ethiopia. Therefore, it could be better source materials for future studies. Nevertheless, there are few drawbacks to this research. First, the cross-sectional nature and Secondly, the consecutive study design of the data may affect the representativeness of the data inference.

**Conclusion:** The study showed that there is a mean difference of liver biomarkers level between diabetic patients with chronic kidney disease and diabetic without chronic kidney disease. The mean value of ALP in T2D patients with CKD was higher when compared with T2D without CKD. However the mean value of both total bilirubin and direct bilirubin were

lower in T2D patients with CKD when compared with those patients without CKD. The double burden of kidney disease upon the hyperglycaemia could aggravate its effect on liver biomarkers. The prevalence of abnormal level of liver enzymes in T2D with CKD was higher when compared T2D without CKD group. This will help to encourage special monitoring and evaluation of such patients for early diagnosis and taking preventive and precautionary measure from developing liver complication.

#### Recommendations

**For Federal Ministry of Health:** Need to design a screening program of liver function test for T2D patients giving special focus to DKD to reduce triple burden.

**For Jimma Medical Center:** Health care providers need to pay attentions on screen all type 2 diabetic patients by assessing liver biomarkers during their initial and follow up visits.

**For Researchers:** Longitudinal studies will be needed to explain a causal relationship between liver biomarkers disorder and risk factors among the type2 diabetes.

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## **Appendix**

### **Annex I**

#### **Consent Form in English**

##### **Purpose**

Diabetic kidney disease is a common incurable chronic disease. During the past decade, DM has emerged as an important clinical and public health problem throughout the world. Likewise, in Ethiopia, diabetes has become more common and it is a fast growing disease. Type 2 diabetes accounts for well over 90% of diabetes in Sub-Saharan Africa. The prevalence of liver diseases is higher in patients who have suffered from DM. Hence, prevention or early diagnosis of progressive liver disease is needed in DM patients. The aim of this studying is to investigate profile of liver biomarkers among diabetic patients with or without CKD who are receiving follow up. Therefore, this study will reflect status of liver among those DM patients with or without CKD.

##### **Participation**

Without your participation, voluntarism and active part of the study the feasibility of the research are under question, so we asking you and all other to voluntary participant in this study. What we expect from you is to be willing to give blood to examine the status of your liver. The examination involves laboratory procedure with collection of 3ml blood from arm. Sample will be collected using sterile and disposable needles and test tubes.

##### **Risks**

Taking 5ml of blood does not have any harm to your health but minor needle pain may last for seconds. If there comes any discomfort, we shall offer you necessary medical treatment freely.

##### **Benefits**

Any status of examination will be communicated with your physician and facilitate appropriate measures need to be taken accordingly.

##### **Confidentiality**

Any information collected from you will be kept confidential. Your identity will not be

disclosed in any situation or study result as we use different code number instead of your name.

### **Sharing the Result**

After analysis of the data, we will present the result of the study to the responsible bodies and may be published in a scientific journal. However, any part of the report will not bear any information about you.

### **Right to Refuse**

Your participation in this study is entirely depending on your decision. You have the right to refuse to accept this request. Your refusal will not affect any part of your treatment in hospital.

### **Contact Address**

If you have any questions or concerns, you can contact Negewo Kebede at any time using the following address Negewo Kebede, Jimma University, Institute of Health, Faculty of medicine, department of biochemical science, medical Biochemistry Unit.

Tel: 0924080567

Email:ngwkebede38@gmail.com

Jimma, Ethiopia

### **Consent Form**

I, the under signed, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind.

I.....do hereby give consent to Dr. /Mr/Mrs /Miss.....to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal is explained to me in the appropriate language I understand.

Unique number of the Participant. ....

Signature of the Participant .....

Name of the Investigator.....

Investigators Signature.....

Date: .....

## Annex II

### Amharic version

የስምምነት ማረጋገጫ ፎርም

#### አላማ

ስኳር በሽታ የማይግል የሚታወቅ በሽታ ነው። በዚህ በ10 ዓመታት በአለማችን ዋነኛው የጤና የሕብረተሰብ ችግር ሆኗል። እንዲሁም በኢትዮጵያ በየጊዜው እያደገ መጥቷል። የ2ኛ ደረጃ ስኳር በሽታ የመካከለኛው አፍሪካ 90 በመቶ ደረጃ ላይ ደርሷል። እነዚህ ስኳር በሽታ የተተኩ ሰዎች በጉበት በሽታ በብዛት ይጠቃሉ። ስለዚህ ይህንን ጉበት በሽታ ሳይባባስ ማከም አስፈላጊ ነው። የዚህ ዋነኛው ጥናት ዓላማ የዚህ ጥናት አላማ የደረጃ ሁለት የስኳር ታማሚ ሆኖ የተለያዩ መድሃኒት የሚወስዱትን የጉበታቸንን ሁኔታ ማጥናት ነው።

#### ተሳትፎ

ይህ ጥናት ያለናንተ ተሳትፎና ፍላጎት ግቡን ይደርሳል ማለት አይቻልም። ስለዚህ ሁላችሁም በዚህ እንዲትሳተፉ እንጠይቃለን። እኛ ከእናንተ የምንፈልገው ፍላጎት የደም ፍራኩስ 5M.L. እንዲትሰጡን

ነው።

#### ጉዳት

5 M.L. የጤና ችግርን ያመጣል ብለን አናምንም ነገር ግን በመርፌው ምክንያት ለትንሽ ሰከንድ ህመም ሊሰማችሁ ይችላል። ሌላ ችግርም ቢፈጠር የሚያስፈልገውን ህክምና በነፃ እንሰጣለን።

#### ፋይዳ

ጥናቱ የሚያሳየውን ውጤት አይተን ሐኪናችሁን ችግር ካለ በአግባቡ እንዲትታከሙ እንጠይቃለን።

#### ሚስጢርን መጠበቅ

ከእናንተ የተሰበሰበው ሁሉ በሚስጢር ይቀመጣል። በስማቸውና በኮድ የያዘው ሚስጢር ካለ አግሞ ተቆልፎበት ይቀመጣል።

#### እንቢ የማለት መብት

ይህ ጥናት በእናንተ ፍላጎት ላይ የተመሰረተ ስለሆነ እንቢ የማለት መብት አላችሁ። ይህ ደግሞ በእናንተ በምታገኙት ሕክምና ላይ ምንም ዓይነት ችግር አያደርስም።

**አድራሻ**

ጥያቄ ወይም ሐሳብ ካላችሁ የፈለጋችሁት ስዓት ደሳለኝ ዳንጎ መገናኘት ትችላላችሁ።

Negewo Kebede : ጅም ዩኒቨርሲቲ ፣ የሕክምና ፋካልቲ ፣ ባዮ ኬሚስትሪ ዲፓርትመንት

ስልክ +2519-24080567

ኢሜል: [ngwkebede38@gmail.com](mailto:ngwkebede38@gmail.com)

ጅም : ኢትዮጵያ

**የቃል ስምምነት**

ከላይ የተፈውን የመረጃ ቅፅ እንብቤሁ የጥናቱን አላማና ጥቅም በግልፅ ተረድቻለሁ።

በዚህም መሰረት ያለ ጥናት ቡድኑ አባላት ተፅኖ በሙሉ ፍቃደኝነት በዚሁ ጥናት ውስጥ

በመሳተፍ የሚጠበቅብኝን አስተዋፅኦ ለማበርከት መወሰኔን በፊርማዬ አረጋግጣለሁ።

የታካሚው መለያ ቁጥር \_\_\_\_\_ ፊርማ \_\_\_\_\_

የመረጃ ሰብሳቢ ሥም \_\_\_\_\_ ፊርማ \_\_\_\_\_

መረጃ የተሰበሰበበት ቀን \_\_\_\_\_ የተጀመረበት ሠዓት \_\_\_\_\_ ያለቀበት ስዓት \_\_\_\_\_

የተቆጣጣሪ ሥም \_\_\_\_\_ ፊርማ \_\_\_\_\_

ቀን \_\_\_\_\_

### **Annex III**

#### **Afan Oromo Version of Consent**

#### **Mirkaneessa Mallattoo Waliigaltee**

#### **Kaayyoo**

Dhibeen sukkaaraa dhibee beekkamaa fi dhukkuba yaala yeroo dheeraa barbaaduu akkasuma irraa fayyuun hin danda'amu. Waggoottan kurnan darban keessa dhibeen sukkaaraa rakkoo cimaa yaala fayyaa fi hawwaasummaa ta'ee Kan addunyaatti as baheedha. Akkuma kan Itoopiyaa keessattis dhibeen kun dhukkuba cimaa fi ariitiidhaan guddachaa jiru ta'ee argamee jira. Biyyoota gammoojjii sahaaraa gadii keessatti gosa dhukkuba kanaa keessaa 90% kan ta'u gosa dhibee sukkaaraa lammaffaadha. Kanaafuu Dhibee tiruu dhukkuba sukkaaraatiin walqabate dursanii addabaasinii beekuu fi ittisuun barbaachisaadha. Kaayyoon qorannoo kanaatiis agarsiistota uumamaan halaa fayyummaa tiruu dhukkubsattoota sukkaaraa kanneen dhukkuba kale yeroo dheeraa qabaniifi hinqabne walibira qabuudaan qorannoo geggeessuudha. Kanaafis qorannoon kun kan agarsiisuuf yaalus haalaa fayyummaa tiruu dhukkubsattoota kanaati.

#### **Hirmaannaa**

Hirmaannaa, fedhii fi gahee keessan malee milkaa'insi qorannoo kanaa deebii hin argatu, kanaafu nutis isiniif warra akka keessan fedhiidhaan hirmaatan ni gaafanna. Wanti nuti isin irraa barbaannuu dhiiga qormaataaf ta'u kennuudhaa haala fayyaa tiruu keessanii qoratamuuf fedhii isin qabdaniidha. Qorannich kan of keessatti qabatu adeemsa 'labooraatoori' dhiiga 5ml irratti geggeeffamuudha. Sumudni kan fudhata marfee qulqullinni isaa mirkanaa'ee fi ergaa itti fayyadamnee booda dhabamsiifamuun fi yeroodhaaf meeshaa kuusa (test tube) ittidha.

#### **Rakkoo Muudachuu Malu**

Dhiiga 5ml fudhachuu miira dhukkubbi iddoo marfeen waraanteetti diqqiqaadhaf dhagahamu

irraa kan hafe miidhaa tokkooyyuu hi qaqqabsiisu. Tarii yoo rakkoon muudates yaala tolaa barbaachisaa ta'e nu biraa argattu.

### **Faayidaa**

Erga qorannoon geggeeffamee boodaa hakima keessan wajjii mari'annee haaluma barbaachisuun yaalli ta'u isiniif godhama.

### **Iccitii Eeguu**

Odeeffannoon sirraa funaaname hundinuu dhoksaan isaa eeggamaadha. Odeeffannoo funaanamu maqaa keetiin otoo hin taane koodiidhan waan ta'eef, haala kammiyyuu keessatti eenyummaan kee nama birootti ifa hin baafamu.

### **Raabsaa Bu'aa Qorannoo**

Bu'aan qorannoo qaama dhimmi ilaallatuuf fi maxansaalee adda addaatiif ni gabaafama. Garuu odeeffannoon kun maqaa keessaniin otoo hin taane kan gabaafamus koodichaana.

### **Mirga Waligaltee Fudhachuu Diduu**

Saba qorannoon kun guutumaanguututti fedhii kee irratti hundaa'eef walii galtee kana dhiisuufis mirgi kee eeggamadha. Diddaan kee yaala ati hospital kana keessatti argattugonkumaa miidhuu hin danda'u.

### **Teessoo**

Gaaffii ykn yaada qabdaniif yeroo barbaaddanitti teessoo armaan gadiitiin nu qunnamuu ni dandeessu

**Nagawoo kabbadaa** Yuunivarsitii Jimmaa Faakaltii Meedikaalaa, Muummee Saayinsii Biomedical.

**Tel. 0924080567**

**Email: [ngwkebede38@gmail.com](mailto:ngwkebede38@gmail.com)**

### **Jimmaa, Itoopiyaa**

### **Uunkaa Waligaltee**

Ani namni maqaa fi mallattoon koo armaan gaditti kaa'ame qorannootti galuuf walgaltee kana kanan kennee kaayyoo fi haala qorannichaa sirritti kanan hubadhe fi yeroon barbaade qorannoo keessa yaada koo geeddarree bahuu akkan danda'u beekeni.

Ani.....an akka yaada kanaatti kan waliigaltee kennu Dr. /obbo/addee.....tiif akka qorannoo kana keessatti hirmaadhuuf.

Ani waayee qorannichaa odeeffannoo barbaachisu hundumaa argadheera akkasums yeroon barbaadetti yaada jijjiiree qorannichaa keessaa adabbii fi bu'aa maleessa otoon hin taane bahuu akkan danda'u mirkaneeffadheera. Karoorri qorannichaas afaan ani beekuun danda'uun

hubachiifameera.

Lakkoofsa addaa hirmaataa.....

Mallattoo hirmaataa.....

Maqaa qorataa.....

Mallattoo qorataa.....

Guyyaa.....

### Annex IV: Sample Collection Sheet

S/No	Sample code	Liver function test(LFTs)					Sex	
		ALT	AST	ALP	TBIL	DBIL	M	F
1	S01							
2	S02							
3	S03							
4	S04							

Sample collection approval

Health professional Name.....

Signature.....

Principal Investigator Name: -Negewo Kebede signature .....

## Annex V: - Questionnaire

Dear respondents, Given below are the items specifying necessary information expected from you. The questionnaire is a part of the study for the masters of degree at Jimma university school of graduate studies. The objective of the research is to investigate profile of liver biomarkers among DM patients with or without CKD who are receiving follow up. This study is purely academic and all your responses will be used in strict confidentiality in accomplishing the requirements of the study. Your genuine answer for the questions in the questionnaire has an immense value to the completion of the study.

Pts clinical Dx _____				
<b>PART 1:- Socio Demographic Information</b>				
No	Question	Code _____	Response	Skip/ Remarks
1.1	Age		<input type="text"/> <input type="text"/> <input type="text"/>	
1.2	Sex		1. Male    2. Female	
1.3	Religion		1. Protestant 2. Orthodox 3. Muslim 4.other-----	
1.4	Level of education		1. Illiterate 2. Can read and write 3. Primary(1-8G) 4.Secondary(9-12G) above	

1.5	Marital status	1. unmarried 2. Married 3. Divorced 4. Widowed	
1.6	Occupation	1. Government employee 2. Housewife 3. Farmer 4. Student 5. Trading 6. Daily laborer 7. Other ---	
1.7	Monthly Income in cash	_____	
<b>PART 2:-Behavioral Measurements</b>			
2.1	Have you ever smoked a part or all of cigarettes?	1. Yes 2. No	
2.2	Have you ever consumed an alcoholic drink such as beer, wine& others?	1. Yes 2. No	
<b>PART 3:- Medically-Related Factors</b>			
3.1	In your family, does anyone have a history of hypertension?	1. Yes 2. No	
3.2	In your family, does anyone have a history of diabetes?	1. Yes 2. No	
3.3	Type of medication for DM		
3.4	Duration of DM on Follow up		
<b>PART 4:- Physical Measurements</b>			
4.1	Height	in (cm) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	
4.2	Weight	in (kg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	
4.3	BMI	In(kg/ (m2) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	
4.4	Waist circumference	in (cm) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	
4.5	Blood Pressure Reading 1	Systolic (mmHg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Diastolic (mmHg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	

4.6	Blood Pressure Reading 2	Systolic (mmHg) <input type="text"/> <input type="text"/> <input type="text"/>	
		Diastolic (mmHg) <input type="text"/> <input type="text"/> <input type="text"/>	
<b>PART 5:- Biochemical Measurements</b>			
5.1	AST	<input type="text"/> <input type="text"/> <input type="text"/>	
5.2	ALT	<input type="text"/> <input type="text"/> <input type="text"/>	
5.3	ALP	<input type="text"/> <input type="text"/> <input type="text"/>	
5.4	TBIL	<input type="text"/> <input type="text"/> <input type="text"/>	
5.5	DBIL	<input type="text"/> <input type="text"/> <input type="text"/>	

**Annex VI: Amharic Version Questionnaire**

ውድ ምላሽ ሰጪዎች፣

ከእርስዎ የሚጠበቁ አስፈላጊ መረጃዎችን የሚገልጹ ዕቃዎች ከዚህ በታች ተሰጥተዋል። መጠይቁ በጅም ዩኒቨርሲቲ የድህረ ምረቃ ትምህርት ቤት የማስተርስ ጥናት አካል ነው። የጥናቱ ዓላማ የዲኤምኤ (CKD) ካላቸው ወይም ከሌላቸው በሽተኞች መካከል ያለውን የጉበት ባዮማርከርን መገለጫ መመርመር ነው። ይህ ጥናት ሙሉ በሙሉ አካዳሚክ ነው እና ሁሉም ምላሾችዎ የጥናቱ መስፈርቶችን ለማሟላት በጥብቅ ሚስጥራዊነት ያገለግላሉ። በመጠይቁ ውስጥ ላሉት ጥያቄዎች እውነተኛ መልስህ ለጥናቱ መጠናቀቅ ትልቅ ዋጋ አለው።

ያላቸው ዲኤም ክሊኒካዊ ምርመራ; _____				
<b>ክፍል 1: - የሶሻል ስነ-ሕዝብ መረጃ</b>				
ተ/ቁ	የጥያቄ ኮድ	ኮድ _____	ምላሽ	አስተያየቶች
1.1	ዕድሜ		<input type="text"/>	
1.2	ፆታ 1. ወንድ 2. ሴት		1. ወንድ 2. ሴት	
1.3	ሃይማኖት		1. ፕሮቴስታንት 2. ኦርቶዶክስ 3. ሙስሊም 4.ሌላ ---	
1.4	የትምህርት ደረጃ		1. መሃይም	

		2. ማንበብ እና መጻፍ ይችላል 3. ዋና (1-8ጂ.) 4. ሁለተኛ (9-12ጂ.) ክላይ	
1.5	የጋብቻ ሁኔታ	1. ያላገባ 2. ያገባ 3. የተፋታ 4. ባል የሞተባት	
1.6	ሥራ	1. የመንግሥት ሠራተኛ 2. የቤት እመቤት 3. ገበሬ 4. ተማሪ 5. ግብይት 6. የቀን ሰራተኛ 7. ሌላ --	
1.7	ወርሃዊ ገቢ በጥሬ ገንዘብ	_____	
<b>ክፍል 2: - የባህሪ መለኪያዎች</b>			
2.1	ከፊል ወይም ሙሉ ሲጋራ አጨስህ ታውቃለህ?	1. አዎ 2. አይ	
2.2	እንደ ቢራ፣ ወይን እና ሌሎች የመሳሰሉ የአልኮል መጠጦችን ጠጥተህ ታውቃለህ?	1. አዎ 2. አይ	ካልሆነ ወደ 2.6 ዝለል
<b>ክፍል 3:- ከህክምና ጋር የተገናኙ ምክንያቶች</b>			
3.1	በቤተሰብዎ ውስጥ የደም ግፊት ታሪክ ያለው ሰው አለ?	1. አዎ 2. አይ	
3.2	በቤተሰብዎ ውስጥ፣ የስኳር ህመም ታሪክ ያለው ሰው አለ?	1. አዎ 2. አይ	
3.3	ለዲኤም መድሃኒት አይነት	መዳኒት _____	
3.4	የዲኤም ቆይታ በክትትል ላይ	_____ አመት/ወር	
<b>ክፍል 4: - አካላዊ መለኪያዎች</b>			
4.1	ቁመት	in (cm) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	

4.2	ክብደት	in (kg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	
4.3	BMI	in (kg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	
4.4	የወገብ ዙሪያ	in (cm) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> . <input type="text"/>	
4.5	የደም ግፊት ንባብ 1	Systolic(mmHg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Diastolic(mmHg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	
4.6	የደም ግፊት ንባብ 2	Systolic (mmHg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> Diastolic(mmHg) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	

## Annex VII: Meeshaa Gaaffilee Qorannoo (Questionnaires)

### Deebistoota Gaaffilee Kaanatif,

Isaan armaan gadii kun odeeffannoo isin irraa barbaannuudha. Meeshaan gaaffiilee qorannichaa qaama qorannoo digrii lammaffaadha Univarsitii Jimmaa mana barnoota qorannoo eebbifamtootati.kaayyoon qorannoo kanaas agarsiistota uumamaan fayyummaa tiruu agarsiisan kanneen dhukkubsattoota dhibee sukkaaraa handuura meedikaala jimmaatti hordoffii taasisaniiti. Qorannoon kun barnoota qofaaf waan ta'eef deebiin keessaniif icciitiin isaa haalaan eegamaadha. Deebiin keessan inni dhugaa kan Gaaffilee meeshaa Gaaffii kana irratti argamaniif kennitan gaheen inni xumura qorannoo kanaa irratti qabu olaanaadha.

Adda baasii dhibee (Dx)				
<b>KUTAA 1 : GAFFILEE HAWWAASUMMAA FI QUUBSAMAAN WALQABATAN</b>				
Lakk.	Gaaffilee	Koodii .....	Deebii	Yaada
1.1	Umrii		<input type="text"/>	
1.2	Saala		1. dhiira 2. Dhalaa	

1.3	Amantii	1. Proteestaantii 2. Ortoodoksii 3. Musliima 4. kan biraa-----	
1.4	Sadarkaa barnootaa	1.Hin baranne 3. dubbiisuu fi Barreessu danda'a 3. kutaa 1-8 barate 4. sad. 2 <sup>ffaa</sup> fi isaa ol	
1.5	Haala gaa'elaa	1. hin fuune/ heerumne 2. fuudhe/heerumte 3.hiike/ hiikte 4. jalaa duute/ du'e	
1.6	Hojii	1.hojjetaa mootummaa 2.haadha manaa 3.qotee bulaa 4. barataa 5.daldalaa 6.dafqaan bulaa 7, kan biraa_____	
1.7	Galii ji'aa qarshiidhaan	_____	
<b>KUTAA 2: SAFARTUU RAKKOO AMALA WAJJIIN WALQABATU</b>			
2.1	Sigaaraa bayyees yaa ta'u xinnoo xuuxxee beektaa?	1.eeyyee 2 . lakki	
2.2	Dhugaatii alkoolii qabu kan akka biraa fi wayinii ni dhugdaa?	1.eeyyee 2 . lakkii	
<b>KUTAA 3: RAKKOO YAALA FAYYAATIIN WALQABATU</b>			
3.1	Maatii keessan keessaa dhiibbaa dhiiga kan dhukkubsate/tu jira?	1. Eeyyee 2. Lakki	
3.2	Maatii keessan keessaa dhukkuba sukkaarra kan dhukkubsate/tu jira?	1. Eeyyee 2. Lakki	
3.3	Qoricha dhukkuba sukkaaraa maal fudhata?	Rx_____	
3.4	Hordoffii Yaala dhukkuba sukkaaraa irra hangam turte?	Waggaa ykn ji'a _____	

KUTAA 4: SAFARTUU QAAMAA			
4.1	Dheerina meetiraan	<input type="text"/> . <input type="text"/>	
4.2	Ulfaatiin/Hanga kiilograaman	<input type="text"/> . <input type="text"/>	
4.3	Marsaa Mudhii	<input type="text"/> . <input type="text"/>	
4.4	Hamma dhiibbaa dhiigaa1	Systolic (mmHg) <input type="text"/> Diastolic (mmHg) <input type="text"/>	
4.5	Hamma dhiibbaa dhiiga 2	Systolic (mmHg) <input type="text"/> Diastolic (mmHg) <input type="text"/>	
4.6	Hamma dhiibbaa dhiiga 3	Systolic (mmHg) <input type="text"/> Diastolic (mmHg) <input type="text"/>	
KUTAA 5: SAFARTUU BIOKEEMIKAALAA			
5.1	AST		
5.2	ALT		
5.3	ALP		
5.4	TBIL		
5.5	DBIL		

## JIMMA UNIVERSITY

### DEPARTMENT OF BIOMEDICAL SCIENCES

#### Declaration

I declare that this thesis paper entitled on **evaluation of Liver Biomarkers and associated among type2 diabetic patients with and T2D without chronic kidney disease attending Jimma Medical Center**. The research is my original work and has not been submitted for a degree in any other university, and all sources of materials used for the research have been properly and suitably acknowledged.

Principal investigator: Negewo Kebede (BSc)

Signature-----

Date-----

This is to certify that the thesis prepared by Negewo Kebede entitled - **Evaluation of Liver Biomarkers and its associated factors among type2 diabetic patients with and without chronic kidney disease attending Jimma Medical Center**. It is Submitted in the Partial Fulfilment of the Requirements for the Degree of Master of Science (Medical Biochemistry) complies with regulation of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Internal Examiner

Name: ..... Signature.....Date.....

External examiner

Name:....., Signature.....Date .....

Advisors

1, Dr. Zerihun Assefa                      Signature.....Date.....

2. Mr. Bati Leta (MSc)                      Signature.....Date.....

3. Mr. Bihonegn Birhan (MSc)              Signature.....Date.....

4. Mr. Abebe Dukessa (MSc)              Signature.....Date.....